The Evergreen Connection of High Performance Separation Techniques with Science and Technology

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Abstract

An ever-increasing number of technological processes and analytical methodologies require the selective separation of chemical compounds occurring in complex mixtures and matrices, either of natural or synthetic origin. Most of the methods employed for this purpose employ high performance separation techniques, which include chromatography, either in gaseous or liquid medium, and a variety of techniques based on the differential migration velocity of the mixture components under the action of either an electric or a gravitational field. These separation techniques comprise electrophoresis, electrochromatography, field-flow fractionation, and ultracentrifugation. Together with chromatography, separation techniques are continuously object of investigations in the area of separation science, aimed at improving separation performance and at developing new applications. The Journal of Chromatography & Separation Techniques provides a forum for reporting and discussing the frequent up-todate of knowledge in separation science and is expected to ensure the needed current information on the recent advancements in this field. Further innovations in high performance separation techniques are expected in all scientific and technological areas. Nevertheless, life science and the quite young -omic sciences, which include genomics, transcriptomics, proteomics, metabolomics, lipidomics, and phytomics are at the forefront. A particularly hot research area is the study of biologically active secondary metabolites occurring in both edible and medicinal plants. Secondary metabolites are known to confer specific sensorial properties and health beneficial effects to plant-derived food products and beverages and have remarkable position as bioactive compounds in medicinal plants, exhibiting numerous biological activities and a variety of health benefits against chronic and degenerative human diseases [1,2]. Important steps for studies aimed at discovering bioactive plant secondary metabolites are the development of methods for the proper sample preparation and extraction, followed by the selective separation of the target compounds, which is performed by suitable instrumental analytical separation techniques, hyphenated to appropriate detectors for identification, chemical characterization, quantification and biological activity evaluation of the isolated compounds [3-5]. Other emerging areas where separation science play a key role include environmental chemistry, nutrigenomics, and personalized medicine, among other significant interdisciplinary research areas occurring at the interfaces between traditional scientific disciplines and technologies. Nevertheless, it is ever needed the development of more efficient and selective methods for the isolation, separation and identification of biological and chemical substances in complex matrices. For example, the quantification of either small or large biomolecules occurring at very low concentration in complex biological and environmental matrices is still a challenging task, which requires the development of novel high performance separation techniques with enhanced sensitivity, either in-line or off-line coupled with highly selective and efficient sample extraction and enrichment methods [6,7]. High demands are also on the use of high performance separation techniques in biomedical sciences. Among others, challenging tasks in this area are the characterization of reliable diagnostic biomarkers for the discovery and implementation of personalized clinical treatments and the accurate identification and quantification of host and viral proteins in cells and extracellular fluids during infections. A remarkable trend that has been interested separation science over the last decades is miniaturization, which has involved both sample extraction and high performance separation techniques. Most of the techniques employed for the removal of potential interferences (sample clean-up), as well as for the extraction and enrichment of the target analytes have been evolved in the corresponding microscale techniques. Parallel to the downsizing of sample extraction and enrichment equipment is the development of miniaturized high performance separation techniques [8]. Common advantages of miniaturized extraction and high performance separation techniques are the possibility to use reduced sample volumes, available in limited quantities for certain application fields (oral sciences, biomedicine, etc.), and the positive impact on both the environment and the analysis costs, due to the reduced consumption and waste of hazardous and expensive chemicals. Other advantages of miniaturization, specific for liquid-phase separation techniques performed in columns of reduced internal diameter (i.d.), are enhanced separation performance, improved heat dissipation through the column (with consequent better temperature control), and higher sensitivity of UV-Vis absorbance detection, own to the lower sample dilution during separation. Moreover, columns used in narrow bore (2.0-3.0 mm i.d.), capillary (0.5-0.1 mm i.d.), and nano (0.15-0.01 mm i.d.) liquid chromatography (LC) are eluted at lower flow rate than traditional analytical size LC columns (4.0-4.6 mm i.d.) and, therefore, are more compatible with mass spectrometry detection, largely employed in LC. The possibility to gather most LC components onto a micro-sized planar structure (microchip) is the results of further technological

advancements. Main advantages of using microchips are the reduced number of hydraulic connections, decreased value of the void volume, reduced band broadening, and possibility to be used as a disposable device with consequent redaction of sample contamination. Another miniaturized high performance separation technique gaining increasing acceptance is capillary electrophoresis (CE), which uses capillary tubes of 0.02-0.10 mm i.d. and, as LC, is performed in a variety of separation modes, including electrochromatography (CEC) that uses packed or monolithic capillary columns similar to those employed in nano LC. The main difference with nano LC is that in CEC the mobile phases is propelled through the capillary column by the electroosmotic flow, instead than a mechanical pump [9]. Comprehensive two-dimensional separation systems have been proposed by coupling LC with CE, although the direct hyphenation of these two techniques requires to overcome a variety of technical problems, including the need of completing the electric circuit for applying the high voltage across the capillary tube used for the CE dimension and the different composition and flow rate of the liquid phases passing throughout the LC and the CE columns [4]. More popular and promising is the use of two-dimensional gas chromatography (GCxGC) and liquid chromatography (LCxLC) separation systems, both hyphenated with mass spectrometry [3, 10]. I hope that this short note would give to the readers of Journal of Chromatography & Separation Techniques the possibility of considering the crucial contribution of separation science to the improvement of knowledge and to the advancements of emerging technologies and scientific investigation areas. Worth of note is the central role played by the large number of high performance separation techniques in accelerating progress on science, technology and global health challenges.

Results:

To demonstrate the gain in peak shape with the CSH column, overloaded elution profiles were recorded on an Atlantis T3 C18 column from Waters with the same dimensions and particle size. Figure 1 compares the elution profiles obtained with the two columns. The peaks obtained on the CSH column are much narrower although the same load is used on both columns and baseline separation could be obtained (Fig. 1b). The difference in chromatographic performance is most pronounced at low TFA concentrations as shown in Fig. 1 for 2.6 mM (0.02%, v/v) TFA. In the following experiments, the TFA concentration was increased to 37.3 mM (0.29%, v/v) to include conditions where the peptide concentration is significantly lower than the TFA concentration, i.e., if competition between TFA and the peptide can be neglected at 37.3 mM TFA it can be neglected also at lower TFA concentrations.